A mathematical approach toward defining and calculating the duration of the lag phase

Plotting the second derivative of a typical bacterial growth curve generates unique maximum and minimum points. The maximum occurs at the end of the lag phase, while the minimum is associated with the end of the exponential growth phase. It appears that the maximum can be used to more accurately define and calculate the duration of the lag phase.

When bacterial cells are transferred from one environment to another, the cultures typically display a period of delay in growth termed the lag phase. During the period, the cells modify their physiological state to take advantage of their new environment and begin replicating. The duration of the lag phase is dependent on an array of factors such as the identity and phenotype of the bacterium, temperature, nutrient availability, pH, and water activity. The lag phase is of particular interest to food microbiologists who attempt to extend the lag phase indefinitely to prevent the growth of spoilage or pathogenic bacteria. A number of different approaches have been used to mathematically define and measure lag phase durations (LPDs), including various pragmatic estimations based on somewhat arbitrary definitions. For example, we (Buchanan and Solberg 1972) have used the time for the initial population density to increase twofold as a definition of the lag phase. An alternate approach that has been used widely is an extrapolation of the portion of the growth curve approximating a linear relationship (i.e. exponential growth phase) back to the initial inoculum level (Fig. 1).

The recent increased availability of computing capability and curve-fitting software has stimulated the use of sigmoidal functions such as the Gompertz and Logistics equations to mathematically depict bacterial growth curves (Gibson et al. 1987, 1988, Buchanan et al. 1989, Zaika et al. 1989). As an example,

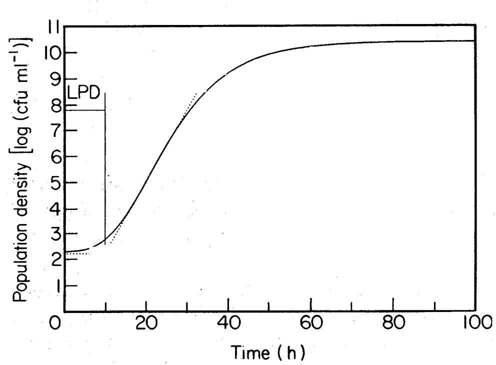

Fig. 1. Example of determination of lag phase duration (LPD) by graphic extrapolation of slope of exponential growth phase to population density at time of inoculation.

Fig. 2 is a curve presented previously by Buchanan and Phillips (1990) that depicts the growth of *Listeria monocytogenes* Scott A in tryptose phosphate broth (Difco) incubated aerobically at 19°C. Plate count data were used to generate the growth curve using the Gompertz equation (Gibson et al. 1987) in conjunction with curve fitting software. The Gompertz equation generates an asymmetrical sigmoid curve based on the relationship,

$$L(t) = A + Ce^{-e^{-[B(t-M)]}}$$

where:

$L(t)$ = log number of bacteria at time t, A = log number of initial level of bacteria, C = log increase in bacterial numbers when culture achieves stationary growth, M = time (h) when culture achieves its maximum growth rate, B = relative growth rate at time M, and T = time.

The LPD can then be estimated using the relationship,

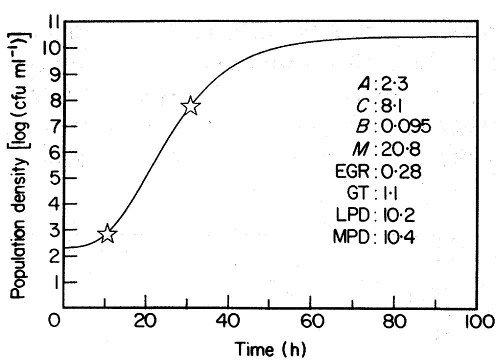

Fig. 2. Growth curve from Buchanan and Phillips (in press) for *Listeria monocytogenes* Scott A cultured aerobically in tryptose phosphate broth at 19°C. Growth curve was generated using 'best-fit' Gompertz equation. Stars indicate times associated with the maximum and minimum values associated with curve depicted in Fig. 4. A, C, B and M are Gompertz parameters (see text). EGR = exponential growth rate, GT = generation time, LPD = lag phase duration, and MPD = maximum population density.

$$\text{LPD} = M - (1/B),$$

which uses the tangent at the maximum absolute growth rate to extrapolate back to lower asymptote to estimate the end of the lag phase in a manner similar to the graphic approach depicted in Fig. 1. The LPD estimated in this manner for the sample growth curve (Fig. 2) was 10·2 h. While the above equation for LPD generally provides reasonable estimates, our experience has indicated that difficulties can occur if the LPD is short and the exponential growth rate is relatively slow. Further, small variations in exponential growth rates tend to produce substantially larger changes in the derived LPD. The purpose of this paper is to propose an alternative mathematical approach to calculating LPDs through the use of the maximum associated with the second derivative of the growth curve.

The Gompertz equation will be used as the basis for further discussions; however, it is important to note that the general approach should be valid for other mathematical models (e.g. logistics equation) used to describe a sigmoidal growth curve. In fact, the original observations concerning the relationships discussed below were based on plotting numerical derivatives (i.e. ΔY and $\Delta[\Delta Y]$) of a hypothetical growth curve without the aid of a mathematical model.

Plotting the rate of change in population density versus time produces a characteristic curve of the type depicted in Fig. 3. Mathematically, this is equivalent to the first derivative of a function describing the growth curve, and Fig. 3 was generated in this manner using the first derivative of the Gompertz equation:

$$\frac{dL(t)}{dt} = CBe^{[-e^{-B(t-M)}-Bt+BM]}.$$

Conceptually, this can be viewed as plotting the 'velocity' of the growth curve.

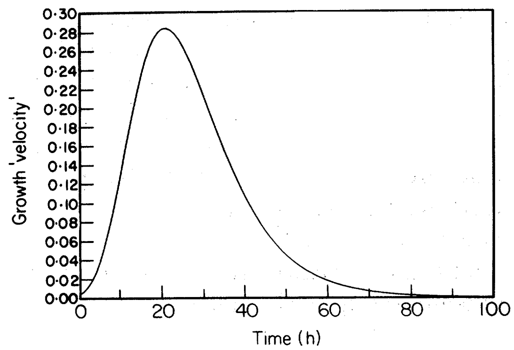

Fig. 3. Plot of the first derivative of the Gompertz equation for the growth curve depicted in Fig. 2.

The maximum generated by this curve is equivalent to the time when the rate of growth is maximal. In the Gompertz model, the maximum occurs at time M.

If the change in the growth velocities vs time is plotted, the culture's growth 'acceleration' can be generated. Alternatively, this can be achieved by finding the second derivative of the initial function. For the Gompertz function, the second derivative is:

$$\frac{d^2L(t)}{dt^2} = CB^2\{e^{[-e^{-B(t-M)}-2B(t-M)]} - e^{[-e^{-B(t-M)}-B(t-M)]}\}.$$

This relationship produces a characteristic curve (Fig. 4) which has distinct maximum and minimum points. The equivalent points on the original growth curve are marked by stars in Fig. 2. The maximum falls at a point coinciding with the end of the lag phase, suggesting that this unique point can be used to non-arbitrarily define the duration of the lag phase. Specifically, the plot suggests that LPD could be defined as the time interval between the inoculation and the attainment of the maximum change in growth rate (i.e. maximum acceleration of the growth curve).

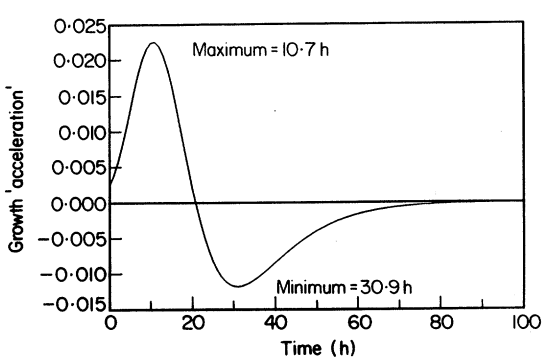

Fig. 4. Plot of the second derivative of the Gompertz equation for the growth curve depicted in Fig. 2.

The minimum associated with the plot of the second derivative (Fig. 4) occurs at a unique point approximating the end of the exponential growth phase (Fig. 2). This suggests that the curve can be used to calculate the duration of the exponential growth phase by determining the difference in time between the minimum and maximum points. The point in time when the second derivative crosses the x-axis (acceleration = 0) is equivalent to the time associated with the maximum in Fig. 3, the time when the growth velocity is maximal.

The times of the maximum and minimum in Fig. 4 can be determined graphically to provide estimates of the durations of the lag and exponential growth phases. Alternatively, the values can be determined mathematically by determining the third derivative of the Gompertz equation and then setting that equal to zero. When this is done, two equations are generated, one for the maximum and the other for the minimum.

Maximum $e^{-B(t-M)} - e^{-(B/2)(t-M)} - 1 = 0.$

Minimum $e^{-B(t-M)} = e^{-(B/2)(t-M)} - 1 = 0.$

These equations can then be solved implicitly, using any of a number of foot finding procedures. In the current example, we used the golden section search method (Bradley et al. 1977), implemented on a microcomputer. The value generated for the LPD using this approach was 10·7 h, compared to the estimated value of 10·2 h obtained by extrapolation of the slope of the exponential growth phase. While the extrapolation method provides a reasonable estimate of LPD, the current approach should supply investigators with a conceptual tool for defining a mathematically unique point for determining this kinetic parameter.

In summary, the current study demonstrated that the second derivative of the growth curve generates a unique value associated with the end of the lag phase. This value can be used to define and calculate the duration of the lag phase. Additionally, the second derivative provides an approach that can be used to define and measure the duration of the exponential growth phase.

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